nature portfolio

Corresponding author(s): Keliang Wu

Last updated by author(s): Nov 23, 2025

Reporting Summary

Nature Portfolio wishes to improve the reproducibility of the work that we publish. This form provides structure for consistency and transparency in reporting. For further information on Nature Portfolio policies, see our Editorial Policies and the Editorial Policy Checklist.

Statistics

For all statistical analyses, confirm that the following items are present in the figure legend, table legend, main text, or Methods section.

n/a	Confirmed	
☐	☒	The exact sample size (*n*) for each experimental group/condition, given as a discrete number and unit of measurement
☐	☒	A statement on whether measurements were taken from distinct samples or whether the same sample was measured repeatedly
☐	☒	The statistical test(s) used AND whether they are one- or two-sided *Only common tests should be described solely by name; describe more complex techniques in the Methods section.*
☐	☒	A description of all covariates tested
☐	☒	A description of any assumptions or corrections, such as tests of normality and adjustment for multiple comparisons
☐	☒	A full description of the statistical parameters including central tendency (e.g. means) or other basic estimates (e.g. regression coefficient) AND variation (e.g. standard deviation) or associated estimates of uncertainty (e.g. confidence intervals)
☐	☒	For null hypothesis testing, the test statistic (e.g. *F*, *t*, *r*) with confidence intervals, effect sizes, degrees of freedom and *P* value noted *Give P values as exact values whenever suitable.*
☒	☐	For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings
☒	☐	For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes
☒	☐	Estimates of effect sizes (e.g. Cohen's *d*, Pearson's *r*), indicating how they were calculated

Our web collection on statistics for biologists contains articles on many of the points above.

Software and code

Policy information about availability of computer code

Data collection	n/a
Data analysis	Smart-seq2 data processing Reads were firstly mapped to mouse (mm10) or human (hg38) genome using STAR (v2.5.3a) and then the quantification results, including raw counts, TPM and FPKM were obtained via RSEM (v1.3.3). For mouse (human), genes with | 〖log〗_2 (fold-change)| > 1 and p-adjust < 0.05 (| 〖log〗_2 (fold-change)| > 0 and p-adjust < 0.1) were identified as DEGs (differentially expressed genes) based on DESeq2 (v1.32.0). Gene function analysis was performed by means of clusterProfiler (v4.2.2). Homer (http://homer.ucsd.edu/homer/motif/) was used for sequence motif enrichment analysis. ULI-NChIP-seq data processing For comparison, raw reads were firstly down-sampled to the same sequencing depth (see Supplemental table S5) and then trimmed using trim_galore (v0.6.7) (https://github.com/FelixKrueger/TrimGalore) with "-q 25 --length 35 -e 0.1 --stringency 4". The clean reads were aligned to mouse genome (mm10) or human genome (hg38) using bowtie2 (v2.2.5), yielding raw bam files, which were then deduplicated using sambamba (v0.6.6). At last, bigwig files with CPM normalization were generated using bamCoverage in deepTools (v3.5.1) for downstream analysis. Peaks of H3K4me3 or H3K27me3 were firstly identified individually using macs2 (v2.2.7.1) and then merged together for differentially analysis conducted in DiffBind (v3.2) (https://github.com/nshanian/DiffBind). Figures exhibiting the distribution of histone signals around specific regions (such as peak center and TSS) were generated using computeMatrix and plotHeatmap in deepTools (v3.5.1). WGBS data processing WGBS data were analyzed following Bismark (v0.23.1) instructions. Briefly, raw reads were firstly trimmed using trim_galore (v0.6.7) (https://

github.com/FelixKrueger/TrimGalore) with "-q 25 --length 35 -e 0.1 --stringency 4" and then mapped to modified mouse (mm10) or human (hg38) genome generated using bismark_genome_preparation. After deduplication, MethylDackel (v0.5.1) (https://github.com/dpryan79/MethylDackel) with default parameters was used to extract DNA methylation information. For the identification of differentially methylated regions (DMR), replicates were firstly merged together and DSS (v2.40.0) (https://github.com/haowulab/DSS) with "delta = 0.1 and p.threshold = 0.001" was further applied. Figures showing the distribution of DNA methylation level around TSS and TES were generated using computeMatrix and plotHeatmap in deepTools (v3.5.1).

scRNA-seq data processing

Raw reads were processed to generate gene expression profiles using CeleScope (v1.15.0, Singleron Biotechnologies) (https://github.com/singleron-RD/CeleScope) with default parameters. Briefly, Barcodes and UMIs were extracted from R1 reads and corrected. Adapter sequences and poly A tails were trimmed from R2 reads and the trimmed R2 reads were aligned against the mouse (mm10) transcriptome using STAR (v2.6.1b). Uniquely mapped reads were then assigned to genes with FeatureCounts (v2.0.1). Successfully Assigned Reads with the same cell barcode, UMI and gene were grouped together to generate the gene expression matrix.

Further analysis based on gene expression matrix was conducted in Seurat (v4.3.0). For each dataset, expression data were filtered using the following criteria: 1) genes expressed in less than 3 cells were excluded; 2) cells with gene features < 50 were excluded; 3) cells with mitochondrial content > 5% were excluded. After filtering, 4357 and 5415 cells, for IVF- and IVO-conceived embryos, respectively, were used for down-stream analysis. After Log-Normalization and scaling, PCA was performed on the scaled data with 2000 highly variable features being used as input. Harmony (v1.2.0) in this study was then applied for data integration and cell clustering was performed using first 40 dimensions with resolution = 1.1. Marker genes were identified and displayed using "FindAllMarkers" in Seurat (v4.3.0) and scRNAtoolVis (v0.1.0) (https://github.com/junjunlab/scRNAtoolVis), respectively. SeuratExtend (v1.1.2) was used for gene function analysis, and developmental trajectories was inferred by means of CytoTRACE2 (v1.0.0) and monocle2 (v2.21.1).

For manuscripts utilizing custom algorithms or software that are central to the research but not yet described in published literature, software must be made available to editors and reviewers. We strongly encourage code deposition in a community repository (e.g. GitHub). See the Nature Portfolio guidelines for submitting code & software for further information.

Data

Policy information about availability of data

All manuscripts must include a data availability statement. This statement should provide the following information, where applicable:

- Accession codes, unique identifiers, or web links for publicly available datasets
- A description of any restrictions on data availability
- For clinical datasets or third party data, please ensure that the statement adheres to our policy

Mouse data related to the comparisons of IVF- and IVO-conceived embryos, including Smart-seq2, H3K27me3 and DNA methylation, have been deposited to the Genome Sequencing Archive (GSA) under the accession number CRA024660. Mouse data related to CPI-455 treatment, including Smart-seq2 and H3K4me3, have been deposited to the GSA under the accession number CRA024689. Human data, including Smart-seq2 with/without CPI-455 treatment, H3K4me3 and DNA methylation, can be accessed from GSA-human under the series number HRA011033.

Research involving human participants, their data, or biological material

Policy information about studies with human participants or human data. See also policy information about sex, gender (identity/presentation), and sexual orientation and race, ethnicity and racism.

Reporting on sex and gender	Sex or gender was not considered in the study design.
Reporting on race, ethnicity, or other socially relevant groupings	The sociopolitical constructs or other socially relevant groupings were not used in this study.
Population characteristics	This was a retrospective cohort study based on data collected from the Reproductive Hospital of Shandong University.This study investigated pregnancy outcomes in women who underwent autologous Day-5 and Day-6 single vitrified blastocyst transfers following IVF/ICSI from January 2020 to December 2023. The inclusion criteria were as follows: 1) patients who underwent their first single embryo thawed transfer at our reproductive center; 2) patients aged between 20 and 45 years. The exclusion criteria included: 1) cervical insufficiency; 2) uterine myomas or malformations; 3) intrauterine adhesion; 4) significant missing data. A total of 16,974 first single frozen-thawed blastocyst transfer cycles were initially identified. Of these, 2,768 cycles were excluded for the following reasons: cervical insufficiency (n=81), uterine myomas (n=1,423), uterine malformations (n=286), intrauterine adhesion (n=965), and significant missing data (n=13). After screening, 14,206 eligible cycles were included in the final analysis. The blastocyst scoring adopted the Gardner and Schoolcraft scoring systems and those with score ≥4BB were determined as high-quality blastocysts.
Recruitment	The human gametes and embryos used in this study were obtained from the Reproductive Hospital of Shandong University. All samples were donated by infertile patients under the premise of fully informed consent. Human immature oocytes were donated by 20-to-38-year-old women with tubal-factor infertility or as the female partners of infertile couples due to male factors, which were clinically discarded during IVF/ICSI treatments and donated by patients after obtaining informed consent.

Ethics oversight	The use of human gametes and embryos in this study complies with all relevant ethical regulations, including the Management of Human Assisted Reproductive Technology (2001), Regulations of Human Assisted Reproductive Technology (2003), Human Biomedical Research Ethics Guidelines(set by National Health Commission of the People's Republic of China on Dec. 1st, 2016), the 2016 Guidelines for Stem Cell Research and Clinical Translation (issued by the International Society for Stem Cell Research, ISSCR) and the Human Embryonic Stem Cell Research Ethics Guidelines (2003), Helsinki Declaration and other laws and regulations. All experiments involving human gamete and embryos were conducted ethically in accordance with the the Reproductive Medicine Ethics Committee of the Reproductive Hospital of Shandong University (2022.12.8).

Note that full information on the approval of the study protocol must also be provided in the manuscript.

Field-specific reporting

Please select the one below that is the best fit for your research. If you are not sure, read the appropriate sections before making your selection.

☒ Life sciences ☐ Behavioural & social sciences ☐ Ecological, evolutionary & environmental sciences

For a reference copy of the document with all sections, see nature.com/documents/nr-reporting-summary-flat.pdf

Life sciences study design

All studies must disclose on these points even when the disclosure is negative.

Sample size	The numbers of embryos used in this study were shown in supplemental table or figure or figure legend.
Data exclusions	No data were excluded from the analyses
Replication	The numbers of biological replicates were displayed as dots in the plot or shown in figure legend.
Randomization	Samples were randomly allocated into experimental groups.
Blinding	The investigators were blinded to group allocation during data collection and analysis.

Reporting for specific materials, systems and methods

We require information from authors about some types of materials, experimental systems and methods used in many studies. Here, indicate whether each material, system or method listed is relevant to your study. If you are not sure if a list item applies to your research, read the appropriate section before selecting a response.

Materials & experimental systems

n/a	Involved in the study
☐	☒ Antibodies
☒	☐ Eukaryotic cell lines
☒	☐ Palaeontology and archaeology
☐	☒ Animals and other organisms
☐	☒ Clinical data
☒	☐ Dual use research of concern
☒	☐ Plants

Methods

n/a	Involved in the study
☐	☒ ChIP-seq
☒	☐ Flow cytometry
☒	☐ MRI-based neuroimaging

Antibodies

Antibodies used	Primary Antibody: anti-CARM1, Mouse, Cell Signaling Technology, 12495S, IF 1:200 anti-OCT4, Mouse, Santa Cruz Biotechnology, sc5729, IF 1:100 anti-CDX2, Rabbit, Abcam, ab76541, IF 1:200 anti-EOMES, Rabbit, Abcam, ab23345, IF 1:100 anti-GATA4, Rabbit, Abcam, ab84593, IF 1:50 anti-TET3, Rabbit, Proteintech, 22612-1-AP, IF 1:200 anti-H3K4me3, Rabbit, Cell Signaling Technology, 9751S, IF 1:200 anti-H3K27me3, Rabbit, Active motif, 39155, IF 1:200 Secondary Antibody: Goat Anti-Mouse IgG H&L (Alexa Fluor 488) preadsorbed, Abcam, ab150117, IF 1:500 Goat Anti-Mouse IgG H&L (Alexa Fluor 594) preadsorbed, Abcam, ab150120, IF 1:500 Goat Anti-Rabbit IgG H&L (Alexa Fluor 488) preadsorbed, Abcam, ab150081, IF 1:500 Goat Anti-Rabbit IgG H&L (Alexa Fluor 647) preadsorbed, Abcam, ab150083, IF 1:500

Validation	Antibodies have been validated in the manuscript.

Animals and other research organisms

Policy information about studies involving animals; ARRIVE guidelines recommended for reporting animal research, and Sex and Gender in Research

Laboratory animals	Female mice (6-8-week-old) and male mice (8-12-week-old) of the Institute for Cancer Research (ICR) were purchased from Beijing Vital River Laboratory Animal Technology Co., Ltd. and reared under specific pathogen-free conditions with a 12-hour light/dark cycle.
Wild animals	This study did not involve wild animals.
Reporting on sex	Sex was not considered in the study design for usage of embryos.
Field-collected samples	This study did not involve field-collected samples.
Ethics oversight	All the experiments carried out in this study were approved by the Ethic Committee of Reproductive Medicine of Reproductive Hospital of Shandong University (2022-138), in accordance with Regulations of the People's Republic of China on Human Assisted Reproductive Technology, the ethical guidelines for Human Assisted Reproductive Technology and Human Sperm Banks, and the Declaration of Helsinki.

Note that full information on the approval of the study protocol must also be provided in the manuscript.

Clinical data

Policy information about clinical studies

All manuscripts should comply with the ICMJE guidelines for publication of clinical research and a completed CONSORT checklist must be included with all submissions.

Clinical trial registration	n/a
Study protocol	n/a
Data collection	n/a
Outcomes	n/a

Plants

Seed stocks	n/a
Novel plant genotypes	n/a
Authentication	n/a

ChIP-seq

Data deposition

☒ Confirm that both raw and final processed data have been deposited in a public database such as GEO.

☒ Confirm that you have deposited or provided access to graph files (e.g. BED files) for the called peaks.

Data access links *May remain private before publication.*	Mouse data generated in this study and related to the comparisons of IVF- and IVO-conceived embryos, including Smart-seq2, H3K27me3 and DNA methylation, have been deposited to the Genome Sequencing Archive (GSA) under the accession number CRA024660 (https://ngdc.cncb.ac.cn/gsa/browse/CRA024660). Mouse data generated in this study and related to CPI-455 treatment, including Smart-seq2 and H3K4me3, have been deposited to the GSA under the accession number CRA024689 (https://ngdc.cncb.ac.cn/gsa/browse/CRA024689). Human data generated in this study, including Smart-seq2 with/without CPI-455 treatment, H3K4me3 and DNA methylation, can be accessed from GSA-human under the series number HRA011033 (https://ngdc.cncb.ac.cn/gsa-human/browse/HRA011033). Source data are provided with this paper.
Files in database submission	human: D51_k4 D52_k4 D53_k4 D61_k4 D62_k4 D63_k4

mouse: F1_K4 F2_K4 O1_K4 O2_K4 C1_K4 C2_K4 F1_K27 F2_K27 O1_K27 O2_K27

Genome browser session
(e.g. UCSC)

https://ngdc.cncb.ac.cn/gsa-human/
https://ngdc.cncb.ac.cn/gsa/

Methodology

Replicates

2 biological replicates for mouse and 3 biological replicates for human

Sequencing depth

Name Total reads Read length
IVF_K4_Rep1 34M 150bp
IVF_K4_Rep2 57.1M 150bp
IVO_K4_Rep1 34.5M 150bp
IVO_K4_Rep2 64.1M 150bp
IVF_K27_Rep1 37.1M 150bp
IVF_K27_Rep2 37.7M 150bp
IVO_K27_Rep1 32.1M 150bp
IVO_K27_Rep2 36.5M 150bp
D5_K4_Rep1 22.8M 150bp
D5_K4_Rep2 32.7M 150bp
D5_K4_Rep3 30.8M 150bp
D6_K4_Rep1 20.7M 150bp
D6_K4_Rep2 19.7M 150bp
D6_K4_Rep3 23.0M 150bp
CPI_K4_Rep1 24.5M 150bp
CPI_K4_Rep2 24.9M 150bp

Antibodies

anti-H3K4me3, Rabbit, Cell Signaling Technology, 9751S
anti-H3K27me3, Rabbit, Active motif, 39155

Peak calling parameters

Peaks of H3K4me3 or H3K27me3 were identified individually using macs2 (v2.2.7.1)

Data quality

We used FastQC (v0.11.9) to evaluate data quality. All samples were in a very good quality call.

Software

ULI-NChIP-seq data processing
For comparison, raw reads were firstly down-sampled to the same sequencing depth (see Supplemental table S5) and then trimmed using trim_galore (v0.6.7) (https://github.com/FelixKrueger/TrimGalore) with "-q 25 --length 35 -e 0.1 --stringency 4". The clean reads were aligned to mouse genome (mm10) or human genome (hg38) using bowtie2 (v2.2.5), yielding raw bam files, which were then deduplicated using sambamba (v0.6.6). At last, bigwig files with CPM normalization were generated using bamCoverage in deepTools (v3.5.1) for downstream analysis. Peaks of H3K4me3 or H3K27me3 were firstly identified individually using macs2 (v2.2.7.1) and then merged together for differentially analysis conducted in DiffBind (v3.2) (https://github.com/nshanian/DiffBind). Figures exhibiting the distribution of histone signals around specific regions (such as peak center and TSS) were generated using computeMatrix and plotHeatmap in deepTools (v3.5.1).